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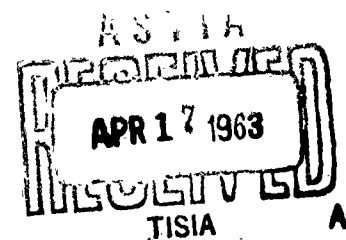
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**TECHNICAL MANUSCRIPT 37**

**FAILURE OF ANIMAL PASSAGE  
TO INCREASE THE VIRULENCE  
OF LISTERIA MONOCYTOGENES**

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# ABSTRACT

Three strains of Listeria monocytogenes, JHH, A4413, and 53P380, were serially passed through mice, guinea pigs, or monkeys via the intracerebral, intraperitoneal and intraspinal routes, respectively. The first two strains had been maintained on laboratory medium for some time; strain 53P380 was received in the brain of a sheep dead of listeriosis and was never inoculated into laboratory media. The virulence of the strains was tested by the intraperitoneal or respiratory route. An apparent increase in virulence for mice of strain JHH was observed following animal passage. However, if the same strain maintained on tryptose agar and brain heart infusion broth was injected in a suspension of normal mouse brain or spleen and liver, there was no difference in the virulence of the strain between animal-passaged and media-grown cells. An adjuvant effect was noted also with broth-grown cells suspended in Bayol F mineral oil or in alcohol or acetone extracts of normal mouse brain. A decrease in virulence for guinea pigs occurred with strain 53P380 after a series of passages by the intraperitoneal route in the same animal.

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## I. INTRODUCTION

The restoration or enhancement of the virulence of microorganisms by passage through susceptible animals has been mentioned by various authors.<sup>1</sup> Jones and Berman<sup>2</sup> described the selection of smooth, virulent strains of Brucella abortus following the inoculation of the less virulent mucoid strains. Braun<sup>3</sup> demonstrated the selection of smooth, virulent Brucella in the presence of serum from susceptible animals but not in the presence of sera from resistant animals. Although increased virulence of Listeria monocytogenes by animal passage has been suggested<sup>4,5</sup> no data seem to be available that indicate changes in virulence after animal passage. Serial passage in mice, guinea pigs, and monkeys was performed in order to follow changes in virulence of organisms maintained in this manner.

## II. MATERIALS AND METHODS

Listeria monocytogenes, strain JHH, isolated from a case of listerial meningitis at The Johns Hopkins Hospital, was received from Dr. L. E. Cluff. Strain A4413 was isolated at the USPHS Communicable Disease Center, Chamblee, Georgia; Dr. C. Olson, Jr. sent us this culture from the University of Wisconsin collection. Strain 53P380 was received from Dr. J. Orsborn of the Colorado State University. It was received in the sheep brain taken at autopsy from a fatal case of listeriosis and was maintained in the brain at -20°C without growth on laboratory media.

Strains JHH and A4413 were cultured in brain heart infusion broth for the first injection of the series. Subsequent passage was in homogenized brain or spleen and liver obtained from moribund animals.

A 16-hour culture of strain JHH was diluted to contain approximately 5 cells per 0.02-ml dose. Mice were injected by the intracerebral route. In two to three days, when the mice appeared moribund, they were sacrificed and brain, spleen, and liver were harvested.\* The organs were ground in a mortar and suspended in sufficient 1.0 per cent tryptose to give a 10.0 per cent suspension (v/v). Spleens and livers were pooled, ground, and suspended together. The suspension was gently centrifuged to remove the larger particles. Two series of passages were maintained: series A consisted of a brain-to-brain passage; in series B the spleen-liver homogenate was injected into the brain. The tissue suspensions were adjusted to contain approximately  $10^3$  cells per dose.

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\* Animals were maintained and used in compliance with the principles established by the National Society for Medical Research, Bio-medical Purview, 1961.



Virulence titrations were performed at intervals, using the tissue suspensions. The bacterial content of the tissue suspension was determined and the desired dilutions prepared in one per cent tryptose; the suspensions were kept at  $-20^{\circ}\text{C}$  when not in use. At the time of injection, the number of organisms in the dilutions was determined again. The Fort Detrick stock of Swiss-Webster mice and Hartley guinea pigs and rhesus monkeys were used. The intraperitoneal or respiratory routes were used for virulence titrations; the latter were performed as described by Roessler and Kautter.<sup>6</sup>

The sheep brain containing strain 53P380 was homogenized and injected directly into mice by the intracerebral route. This strain was passed 24 times through mice and then through guinea pigs; the pigs were infected by the intraperitoneal route.

L. monocytogenes, strain A4413, was used for both a series of intraspinal passages through rhesus monkeys and for respiratory passage through mice. After the first intraspinal injection of the brain-heart infusion culture, the inoculum for monkey passage was a brain suspension from the previous passage. The inocula varied from  $2.8 \times 10^3$  to  $5.6 \times 10^7$  cells. For the initial respiratory exposure in mice, slightly more than the respiratory LD<sub>50</sub> ( $3.5 \times 10^3$  cells) was administered. The animals were sacrificed just before death and the spleens and livers were homogenized together and used for the succeeding passage. In order to obtain a tissue suspension satisfactory for passage through the nozzle of the Collison spray device,<sup>6</sup> the tissue was first mixed in a Waring blender with sand and 0.1 per cent tryptose in saline. The homogenate was then centrifuged in an International PR2 centrifuge at 1100 revolutions per minute for 60 minutes. The decanted supernatant fluid was filtered two times through a coarse sintered-glass filter and then two times through a medium filter. The filters were washed each time with tryptose saline. Suspensions prepared in this way contained only particles capable of passage through the spray device.

### III. RESULTS

Table I indicates an apparent tenfold increase in the virulence of L. monocytogenes, strain JHH, passed five or more times through mice when compared with the same strain maintained on laboratory medium. However, when the cells of broth-grown Listeria were suspended in a 10 per cent brain homogenate from normal mice, a similar decrease in the LD<sub>50</sub> was observed. Suspension of L. monocytogenes in Bayol F, a light mineral oil, or in the acetone or alcohol extract of normal mouse brain also reduced the number of organisms necessary to kill 50 per cent of the animals within 15 days. The LD<sub>50</sub> for the broth suspension was  $3.4 \times 10^4$  cells; for the cell suspension in oil, it was only  $5.4 \times 10^2$  cells; for the two brain extracts, the LD<sub>50</sub> was less than  $1 \times 10^3$  cells. When the brain suspension of the tenth passage

TABLE I. VIRULENCE FOR MICE OF LISTERIA MONOCYTOGENES  
MAINTAINED BY SERIAL PASSAGE IN MICE

Number of Passages	LD <sub>50</sub> , cells			Control in Suspension of Normal Mouse Tissue
	Series A <sup>a</sup> /	Series B <sup>b</sup> /	Control <sup>c</sup> /	
5	1.9 x 10 <sup>4</sup>	3.6 x 10 <sup>4</sup>	2.7 x 10 <sup>5</sup>	1.2 x 10 <sup>4</sup>
5				9.0 x 10 <sup>4</sup>
10	2.5 x 10 <sup>4</sup>		1.5 x 10 <sup>6</sup>	8.3 x 10 <sup>3</sup>
35	7.1 x 10 <sup>4</sup>	3.6 x 10 <sup>4</sup>		
57	1 x 10 <sup>4</sup>	3.8 x 10 <sup>4</sup>	5.2 x 10 <sup>4</sup>	

a. Brain-to-brain passage.

b. Infected spleen and liver tissue injected into brain.

c. Grown in brain heart infusion broth at 37°C for 16 hours.

was used as an inoculum for a single passage through brain heart infusion broth, the intraperitoneal LD<sub>50</sub> for this culture was  $3.4 \times 10^5$  cells. This was not significantly different at the 95 per cent level from the LD<sub>50</sub> for the culture maintained continuously on laboratory media ( $1.5 \times 10^6$  cells with 95 per cent confidence limits of  $3.4 \times 10^5$  to  $6.1 \times 10^6$  cells). After 57 passages no alteration in virulence was observed and the experiment was concluded.

The intraperitoneal LD<sub>50</sub> for L. monocytogenes, 53P380, for mice decreased from  $1.7 \times 10^4$  cells to  $1.5 \times 10^2$  cells after 24 intracranial passages in mice plus 16 passages through guinea pigs by the intraperitoneal route. It is likely that this decrease was also the result of the homogenized tissue, since there was no alteration of the respiratory LD<sub>50</sub> for mice.

During the intraperitoneal passage in guinea pigs there appeared to be a decrease rather than an increase in virulence. The incubation period became longer and at autopsy the number of Listeria recovered from the tissues decreased. Following the eighth serial passage the pigs were apparently healthy at 14 days and no organisms were recovered from the tissues upon autopsy and culture. The virulence of this strain in the original sheep brain was compared with that in mouse brain after 24 intracranial passages and with the organisms in the spleen-liver suspension from the eleventh guinea pig passage via the peritoneal cavity. The LD<sub>50</sub> values were  $2.7 \times 10^6$ ,  $1.7 \times 10^6$ , and  $5.0 \times 10^6$  respectively.

L. monocytogenes, strain A4413, passed through monkeys 22 times by intraspinal injection also failed to show an alteration in virulence. No deaths resulted among the six monkeys exposed to doses ranging from  $10^7$  to  $10^8$  cells by the respiratory route. Fever, positive blood cultures during the first 24 hours, and the formation of antibodies against Listeria indicated that an infection had been established. However, similar findings were obtained with this strain maintained on laboratory media.

#### IV. DISCUSSION

It is apparent from these results that no increase in virulence occurred following rather extensive animal passage of strains of Listeria. Enhancement occurred for neither the host species used for passage nor for other animal species. Owens et al<sup>7</sup> experienced a similar failure to increase the virulence of Pasteurella tularensis by passage through laboratory animals. These same authors were unable to detect any increase in virulence of P. tularensis during the course of several natural epizootics.

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